Cont A'

XbaI and PstI sites respectively. This entire construct was then ligated into KpnI/HindIII digested pUC19. The inclusion of a T7 promoter sequence and a HgaI site flanking the trailer and leader sequences, respectively, allowed in vitro synthesis of RSV/CAT RNA transcripts containing the precise genomic sequence 3' and 5' ends.--

On page 7, please replace the paragraph beginning, "FIG. 3. Schematic representation of the RSV strain A2 genome..." with the following paragraph:

A 2

--FIG. 3. Schematic representation of the RSV strain A2 genome showing the relative positions of the primer pairs used for the synthesis of cDNAs comprising the entire genome. The endonuclease sites used to splice these clones together are indicated; these sites were present in the native RSV sequence and were included in the primers used for cDNA synthesis. Approximately 100 ng of viral genomic RNA was used in RT/PCR reactions for the separate synthesis of each of the seven cDNAs. The primers for the first and second strand cDNA synthesis from the genomic RNA template are also shown. For each cDNA, the primers for the first strand synthesis are nos. 1-7 (SEQ ID NOs:43-49) and the primers for the second strand synthesis are nos. 1'-7 '(SEQ ID NOs:50-56).--

On page 8, please replace the paragraph beginning, "FIG. 4. Schematic representation of the RSV subgroup B strain B9320..." with the following paragraph:

A 3

--FIG. 4. Schematic representation of the RSV subgroup B strain B9320. BamH1 sites were created in the oligonucleotide primers (SEQ ID NOs:57 and 58) used for RT/PCR in order to clone the G and F genes from the B9320 strain into RSV subgroup A2 antigenomic cDNA (FIG. 4A). A cDNA fragment which contained G and F genes from 4326 nucleotides to 9387 nucleotides of A2 strain was first subcloned into pUC19 (pUCRVH). Bgl II sites were created at positions of 4630 (SH/G intergenic junction) (FIG. 4B) and 7554 (F/M2 intergenic junction (FIG. 4C). B93260 A-G and -F cDNA inserted into pUCR/H which is deleted of the A-G and F genes. The resulting antigenomic cDNA clone was termed as pRSVB-GF and was used to transfect Hep-2 cells to generate infectious RSVB-GF virus.--

On page 9, please replace the paragraph beginning,"FIG. 10. RSV L protein charged residue clusters targeted for site-directed mutagenesis..." with the following paragraph:

a4

--FIG. 10. RSV L protein (SEQ ID NO: 59) charged residue clusters targeted for site-directed mutagenesis. Contiguous charged amino acid residues in clusters were converted to alanines by site-directed mutagenesis of the RSV L gene using the QuikChange site-directed mutagenesis kit (Stratagene).--

On page 9, please replace the paragraph beginning,"FIG. 11. RSV L protein cysteine residues targeted for site-directed mutagenesis..." with the following paragraph:

a5

--FIG. 11. RSV L protein (SEQ ID NO: 59) cysteine residues targeted for site-directed mutagenesis. Cysteine residues were converted to alanine-residues by site-directed mutagenesis of the RSV L gene using the QuikChange site-directed mutagenesis kit (Stratagene).--

On page 10, please replace the paragraph beginning,"FIG. 13. Structure of rA2ΔM2-2 genome..." with the following paragraph:

a6

--FIG. 13. Structure of rA2ΔM2-2 genome and recovery of rA2ΔM2-2. (A). Sequences shown is the region of the M2 gene that M2-1 and M2-2 open reading frames overlap (SEQ NOs: 60-62). Total of 234 nt that encode the C-terminal 78 amino acids of M2-2 was deleted through the introduced *Hind III* sites (underlined)(SEQ NOs: 63 and 64). The N-terminal 12 amino acid residues of the M2-2 open reading frame are maintained as it overlaps with the M2-1 gene. (B). RT/PCR products of rA2ΔM2-2 and rA2 viral RNA using primers V1948 and V1581 in the presence (+) or absence (-) of reverse transcriptase (RT). The size of the DNA product derived from rA2 or rA2ΔM2-2 is indicated.--

On page 12, please replace the paragraph beginning,"Fig.26. Insertion of the G and F genes..." with the following paragraph:

a7

--Fig.26. Insertion of the G and F genes of RSV B9320 strain into recombinant A2 strain. The G and F genes of B9320 were amplified by RT/PCR using primers that contained the BamH I restriction enzyme sites (SEQ NOs: 65 and 66). A DNA cassette containing the G and F genes of B9320 was then introduced into the

pRSV(R/H) subclone using the introduced Bgl II restriction enzyme sites that flanked the RSV G and F genes of the A2 strain. The cDNA fragment containing the G and F genes of B9320 was subsequently shuffled into the full-length A2 antigenomic cDNA by ligating at the Xho I and BamH I sites. The gene start signal of the G gene and the gene end signal of the F gene of B9320 are underlined and the restriction enzyme sites used for cloning are indicated.--

On pages 35 through 36, please replace the paragraph beginning, "The cDNAs of the 44 nucleotide leader and 155 nucleotide trailer..." with the following paragraph:

--The cDNAs of the 44 nucleotide leader and 155 nucleotide trailer components of RSV strain A2 (see Mink et al., Virology 185:615-624 (1991); Collins et al., Proc. Natl. Acad. Sci. 88:9663-9667 (1991)), the trailer component also including the promoter consensus sequence of bacteriophage T7 polymerase, were separately assembled by controlled annealing of oligonucleotides with partial overlapping complementarity (see Fig. 1). The oligonucleotides used in the annealing were synthesized on an Applied Biosystems DNA synthesizer (Foster City, CA). The separate oligonucleotides and their relative positions in the leader and trailer sequences are indicated in Fig. 1. The oligonucleotides used to construct the leader were:

1. 5'CGA CGC ATA TTA CGC GAA AAA ATG CGT ACA ACA AAC TTG CAT AAA C (SEQ ID NO: 1)
2. 5'CAA AAA AAT GGG GCA AAT AAG AAT TTG ATA AGT ACC ACT TAA ATT TAA CT (SEQ ID NO: 2)
3. 5'CTA GAG TTA AAT TTA AGT GGT ACT (SEQ ID NO: 3)
4. 5'TAT CAA ATT CTT ATT TGC CCC ATT TTT TTG GTT TAT GCA AGT TTG TTG TA (SEQ ID NO: 4)
5. 5'CGC ATT TTT TCG CGT AAT ATG CGT CGG TAC (SEQ ID NO: 5)--

On page 36, please replace the paragraph beginning, "The oligonucleotides used to construct the trailer were: ..." with the following paragraph:

-- The oligonucleotides used to construct the trailer were:

1. 5'GTA TTC AAT TAT AGT TAT TAA AAA TTA AAA ATC ATA TAA TTT TTT AAA TA (SEQ ID NO: 6)
2. 5'ACT TTT AGT GAA CTA ATC CTA AAG TTA TCA TTT TAA TCT TGG AGG AAT AA (SEQ ID NO: 7)
3. 5'ATT TAA ACC CTA ATC TAA TTG GTT TAT ATG TGT ATT AAC TAA ATT ACG AG (SEQ ID NO: 8)
4. 5'ATA TTA GTT TTT GAC ACT TTT TTT CTC GTT ATA GTG AGT CGT ATT A (SEQ ID NO: 9)
5. 5'AGC TTA ATA CGA CTC ACT ATA ACG A (SEQ ID NO: 10)
6. 5'GAA AAA AAG TGT CAA AAA CTA ATA TCT CGT AAT TTA GTT AAT ACA CAT AT (SEQ ID NO: 11)
7. 5'AAA CCA ATT AGA TTA GGG TTT AAA TTT ATT CCT CCA AGA TTA AAA TGA TA (SEQ ID NO: 12)
8. 5'ACT TTA GGA TTA GTT CAC TAA AAG TTA TTT AAA AAA TTA TAT GAT TTT TA (SEQ ID NO: 13)
9. 5'AATT TTT AAT AAC TAT AAT TGA ATA CTG CA (SEQ ID NO: 14) --

On page 39, please replace the paragraph beginning, "The following oligonucleotides were used to construct the ribozyme/T7 terminator sequence: ..." with the following paragraph:

-- The following oligonucleotides were used to construct the ribozyme/T7 terminator sequence:

5'GGTGGCCGGCATGGTCCCAGC
3'CCA CCGGCCGTACCAGGGTCG
(SEQ ID NO: 15)

CTCGCTGGCGCCGGCTGGGCAACA
GAGCGACCGCGGCCGACCCGTGTG
(SEQ ID NO: 16)

TTCCGAGGGGACCGTCCCCTCGGT

(cont'd)

AAGGCTCCCCTGGCAGGGGAGCCA
(SEQ ID NO: 17)

AATGGCGAATGGGACGTCGACAGC
TTACCGCTTACCCTGCAGCTGTCG
(SEQ ID NO: 18)

TAACAAAGCCCGAAGGAAGCT
ATTGTTTCGGGCTTCCTTCGA
(SEQ ID NO: 19)

GAGTTGCTGCTGCCACCGTTG
CTCAACGACGACGGAGGCAAC
(SEQ ID NO: 20)

AGCAATAACTAGATAACCTTGGG
TCGTTATTGATCTATTGGAACCC
(SEQ ID NO: 21)

CCTCTAAACGGGTCTTGAGGGTCT
GGAGATTTGCCCAGAACTCCCAGA
(SEQ ID NO: 22)

TTTTGCTGAAAGGAGGAACTA
AAAACGACTTTCCTCCTTGAT
(SEQ ID NO: 23)

TATGCGGCCGCGTCGACGGTA
ATACGCCGGCGCAGCTGCCAT
(SEQ ID NO: 24)

CCGGGCCCGCCTTCGAAG3'

Cont A10

GGCCCGGGCGGAAGCTTC5'
~~(SEQ ID NO: 25)~~

On pages 52 through 53, please replace the paragraph beginning, "Recombinant RSVB-GF virus was characterized by RT/PCR..." with the following paragraph:

A11

-- Recombinant RSVB-GF virus was characterized by RT/PCR using RSV subgroup B specific primers. Two independently purified recombinant RSVB-GF virus isolates were extracted with an RNA extraction kit (Tel-Test, Friendswood, TX) and RNA was precipitated by isopropanol. Virion RNAs were annealed with a primer spanning the RSV region from nt 4468 to 4492 and incubated for 1 hr under standard RT conditions (10 µl reactions) using superscript reverse transcriptase (Life Technologies, Gaithersburg, MD). Aliquots of each reaction were subjected to PCR (30 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 2 min) using subgroup B specific primers in G region (CACCACCTACCTTACTCAAGT (SEQ ID NO: 26) and TTTGTTTGTGGGTTTGATGGTTGG (SEQ ID NO: 27)). The PCR products were analyzed by electrophoresis on 1% agarose gel and visualized by staining with ethidium bromide. As shown in Fig. 5, no DNA product was produced in RT/PCR reactions using RSV A2 strain as template. However, a predicted product of 254 bp was detected in RT/PCR reactions utilizing RSVB-GF RNA or the PCR control plasmid, pRSVB-GF DNA, as template, indicating the rescued virus contained G and F genes derived form B9320 virus. --

On page 53, please replace the paragraph beginning, "RSV subgroup B strain B9320 G gene was amplified from B9320 vRNA..." with the following paragraph:

A12

-- RSV subgroup B strain B9320 G gene was amplified from B9320 vRNA by RT/PCR and cloned into pCRII vector for sequence determination. Two Bgl II sites were incorporated into the PCR primers which also contained gene start and gene end signals (GATATCAAGATCTACAATAACATTGGGGCAAATGC (SEQ ID NO: 28) and GCTAAGAGATCTTTTT GAATAACTAAGCATG (SEQ ID NO: 29)). B9320G cDNA insert was digested with Bgl II and cloned into the SH/G (4630 nt) or F/M2 (7552 nt) intergenic junction of a A2 cDNA subclone (Fig. 4B and Fig. 4C). The Xho I to Msc I fragment containing B9320G insertion either at SH/G or F/M2

Cont A12

intergenic region was used to replace the corresponding Xho I to Msc I region of the A2 antigenomic cDNA. The resulting RSV antigenomic cDNA clone was termed as pRSVB9320G-SH/G or pRSVB9320G-F/M2.--

On page 54, please replace the paragraph beginning, "Expression of the inserted RSV B9320 G gene was analyzed by Northern blot..." with the following paragraph:

A13

-- Expression of the inserted RSV B9320 G gene was analyzed by Northern blot using a ^{32}P-labeled oligonucleotide specific to A2-G or B-G mRNA. Total cellular RNA was extracted from Hep-2 cells infected with wild-type RSVB 9320, rRSVA2, or rRSVB9320G-F/M2 48 hours postinfection using an RNA extraction kit (RNA stat-60, Tel-Test). RNA was electrophoresed on a 1.2% agarose gel containing formaldehyde and transferred to a nylon membrane (Amersham). An oligonucleotide specific to the G gene of the A2 stain (5'TCTTGACTGTTGTGGATTGCAGGGTTGACTTGACTCCGATCGATCC-3' (SEQ ID NO: 30)) and an oligonucleotide specific to the B9320 G gene (5'CTTGTGTTGTTGTTGTATGGTGT GTTTCTGATTTTGTATTGATCGATCC-3' (SEQ ID NO:31)) were labeled with ^{32}P-ATP by a kinasing reaction known to those of ordinary skill in the art. Hybridization of the membrane with one of the ^{32}P-labeled G gene specific oligonucletodies was performed at 65°C and washed according to standard procedure. Both A2-G and B9320-G specific RNA were detected in the rRSVB9320G-F/M2 infected Hep-2 Cells. (Figure 6B) These results demonstrate subtype specific RNA expression.--

On page 101, please replace the table labeled, "Table 18. Primers used for changing each cysteine codon in the M2-1 gene" with the following table:

A14

Table 18. Primers used for changing each cysteine codon in the M2-1 gene[a]

Primer	Position in RSV antigenome	Sequence
MC1	nt 7609-7641	5'TCACGAAGGAATCCTGGCAAATTTGAAATT CGA (SEQ ID NO: 32)
MC2	nt 7633-7665	5'GAAATTCGAGGTCATGGTTTAAATGGTAA GAGG (SEQ ID NO: 33)

MC3	nt 7648-7683	5'TGCTTAAATGGTAAGAGGGGACATTTTAGT CATAAT (SEQ ID NO: 34)
MC4	nt 7876-7908	5'ACTAAACAATCAGCAGGTGTTGCCATGAG CAAA (SEQ ID NO: 35)

[a] The numbers correspond to nucleotides in the RSV antigenome. Nucleotides that were mutated to change cysteine codons to glycine codons are in bold and underlined.

On page 104, please replace the table labeled, "Table 20. Primers used to introduce tandem termination codons in the C-terminus of the M2-1 protein" with the following table:

Table 20. Primers used to introduce tandem termination codons in the C-terminus of the M2-1 protein

Primer	Position in RSV antigenome	Sequence[a]
MSCH 1	nt 7960-8011	5'GAGCTAAATTCACCCAAGAT*AAGCTT*GT**TAATAA** ACTGTCATATCATATATTG (SEQ ID NO: 36)
MSCH2	nt 8035-8076	5' CAAACTATCCATCTG**TAATA***AAGCTT*GCCAGCA GACGTATTG (SEQ ID NO: 37)
MSCH3	nt 8120-8169	5' CCATCAACAACCCAAAA**TAATA***AAGCTT*TAGTG ATACAAATGACCATGCC (SEQ ID NO: 38)

[a] The numbers correspond to nucleotides in the RSV antigenome. Tandem stop codons are indicated in bold. Mutated nucleotides are underlined and unique Hind III sites introduced simultaneously with the tandem stop codon are shown in italics.

On page 106, please replace the paragraph beginning, "The wild type RSV B9320 was grown in Vero cells ..." with the following paragraph:

-- The wild type RSV B9320 was grown in Vero cells and the viral RNA was extracted from infected cell culture supernatant. A cDNA fragment containing the G and F genes of RSV B9320 was obtained by RT/PCR using the following primers: ATCA*GGATCC*ACAATAACATTGGGGCAAATGCAACC (SEQ ID NO: 39) and CTGGCATTC*GGATCC*GTTTTATGTAACTATGAGTTG (SEQ ID NO: 40) (the BamH I sites engineered for cloning is in italics and B9320 specific sequences are underlined). BamH I restriction enzyme sites were introduced upstream of the gene start sequence of G and downstream of the gene end sequence of F. The PCR product

was first introduced into the T/A cloning vector (Invitrogen) and the sequences were confirmed by DNA sequencing. The BamH I restriction fragment containing the G and F gene cassette of B9320 was then transferred into a RSV cDNA subclone pRSV(R/H) that contained RSV sequences from nt 4326 to nt 9721 through the introduced Bgl II sites at nt 4655 (upstream of the gene start signal of G) and at nt 7552 (downstream of the gene end signal of F). Introduction of these two Bgl II sites were made by PCR mutagenesis using the QuickChange mutagenesis kit (Strategene, La Jolla, CA). BamH I and Bgl II restriction enzyme sites have compatible ends but ligation obliterates both restriction sites. The Xho I (nt 4477) to BamH I (nt 8498) restriction fragment containing the G and F genes of B9320 was then shuttled into the infectious RSV antigenomic cDNA clone pRSVC4G (Jin et al., 1998). The chimeric antigenomic cDNA was designated pRSV-G_BF_B. To delete the M2-2 gene from pRSV-G_BF_B, the Msc I (nt 7692) to BamH I (nt 8498) fragment from rA2ΔM2-2 which contained the M2-2 deletion (Jin et al., 2000a) was introduced into pRSV-G_BF_B. The chimeric cDNA clone that lacks the M2-2 gene was designated pRSV-G_BF_BΔM2-2. --

On page 107, please replace the paragraph beginning, "-The expression of viral RNA for each recovered chimeric RSV was analyzed ..." with the following paragraph:

--The expression of viral RNA for each recovered chimeric RSV was analyzed by Northern blotting. Total cellular RNA was extracted from virus infected cells at 48 hr post-infection. The RNA blot was hybridized with a γ-^{32}P-ATP labeled oligonucleotide probe specific for the F gene of B9320 (GAGGTGAGGTACAATGCATTAATAGCAAGATGGAGGAAGA (SEQ ID NO: 41)) or a γ-^{32}P-ATP labeled probe specific for the F gene of A2 (CAGAAGCAAAACAAAATGTGACTGCAGTGAGGATTGTGGT (SEQ ID NO: 42)). To detect the G mRNA of the chimeric viruses, RNA blots were hybridized with a 190-nt riboprobe specific to the G gene of B9320 or a 130nt riboprobe specific to the G gene of A2. Both riboprobes were labeled with α-^{32}P-CTP. Hybridization was performed at 65°C in Express Hyb solution (Clontech, Palo Alto, CA) overnight. Membranes were washed at 65°C under stringent condition and exposed to film.--